Effects of multiple predator species on green treefrog (*Hyla cinerea*) tadpoles

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Abstract: Prey species that occur across a range of habitats may be exposed to variable communities of multiple predator species across habitats. Predicting the combined effects of multiple predators can be complex. Many experiments evaluating the effects of multiple predators on prey confound either variation in predator density with predator identity or variation in relative predator frequency with overall predation rates. We develop a new experimental design of factorial predator combinations that maintains a constant expected predation rate, under the null hypothesis of additive predator effects. We implement this design to evaluate the combined effects of three predator species (bass, aeshnid and libellulid odonate naiads) on mortality rate of a prey species, *Hyla cinerea* (Schneider, 1799) tadpoles, that occurs across a range of aquatic habitats. Two predator treatments (libellulid and aeshnid + libellulid) resulted in lower tadpole mortality than any of the other predator treatments. Variation in tadpole mortality across treatments was not related to coarse variation in microhabitat use, but was likely due to intraguild predation, which occurred in all predator treatments. *Hyla cinerea* tadpoles have constant, low survival values when exposed to many different combinations of predator species, and predation rate probably increases linearly with predator density.

Résumé : Les espèces de proies qui vivent dans une gamme d'habitats différents peuvent être exposées dans ces milieux à des communautés variables d'espèces multiples de prédateurs. La prédiction des effets de prédateurs multiples peut ainsi être compliquée. Plusieurs des expériences qui cherchent à évaluer les effets des prédateurs multiples sur les proies confondent la variation dans la densité des prédateurs et l'identité des prédateurs ou alors la variation de la fréquence relative des prédateurs et les taux globaux de prédation. Nous avons mis au point un nouveau plan d'expérience factoriel de combinaisons de prédateurs qui maintient un taux prévu de prédation constant, avec une hypothèse nulle d'effets cumulatifs des prédateurs. Nous utilisons ce plan d'expérience pour évaluer les effets combinés de trois prédateurs (achigan, larves d'odonates aeshnidés et libellulidés) sur le taux de mortalité d'une espèce de proies, des têtards d'*Hyla cinerea* (Schneider, 1799), qui se retrouvent dans une variété d'habitats. Deux conditions expérimentales de prédation (libellulidés et combinaison d'aeshnidés et de libellulidés) causent une mortalité plus basse des têtards que toutes les autres conditions. La variation de la mortalité des têtards dans les différentes conditions expérimentales n'est pas reliée à la variation grossière de l'utilisation des microhabitats, mais est vraisemblablement due à la prédation à l'intérieur des guildes qui se produit dans toutes les conditions de prédation. Les têtards d'*H. cinerea* possèdent des taux de survie faibles et constants lorsqu'on les expose à différentes combinaisons d'espèces prédatrices et les taux de prédation augmentent probablement de façon linéaire en fonction de la densité des prédateurs.

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Introduction

Although most prey species probably encounter multiple predator species in nature, the effects of multiple predator species on prey mortality are less studied than the effects of single predator species (Sih et al. 1998). Prey species that occur across a habitat gradient might be expected to have adaptations to cope with many different combinations of multiple predator species (Hopper 2001). Multiple predators have additive effects on prey if their combined effects can be predicted by knowing their effects on prey individually (Travis et al. 1985; Sokol-Hessner and Schmitz 2002); alternatively, their combined effects may be greater or less than additive

(Werner and McPeek 1994; Crowder et al. 1997; Vonesh and Osenberg 2003; Siddon and Witman 2004).

Non-additive effects of multiple predators may be due to attributes of prey, predators, or both. Effects of combined predators can be greater than expected if an antipredator behavior that is effective against one species dramatically increases the predation rate from the other species (Soluk 1993). Predator interactions can also result in greater than additive effects if the reduction in prey density produced by one predator elicits increased foraging behavior by the other (Soluk 1993). Alternatively, the combined effects of predators may be less than additive if prey tactics are effective against both predator types (Crowder et al. 1997). Intraguild

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predation can produce a combined predator effect that is less than additive, since predators reduce their own total density by treating one another as alternative prey (Holt and Polis 1997; Snyder and Ives 2001).

Effects of multiple predator species on prey species have been studied using several designs. The most common design uses a density series of each predator in a factorial design (Sih et al. 1998). This design confounds total predator density with predator composition and can complicate the interpretation of results. An alternative design maintains a constant total density of predator individuals across treatments of all possible predator combinations (Sokol-Hessner and Schmitz 2002). This substitutive design allows the effects of predator identity to be distinguished from the effects of increased total predator density. However, if predator species vary widely in their per capita predation rates, this design will confound the relative frequency of different predator species with variation in total attack rate.

Here we present a study of how three predators (bass, aeshnid, and libellulid odonate naiads) combine to affect survival of *Hyla cinerea* (Schneider, 1799) tadpoles. *Hyla cinerea* occurs across a habitat gradient with variation in predation pressure (Gunzburger and Travis 2004), thus it is an ideal prey species in which to study the effects of different combinations of predator species. Our experimental design is a variation on the substitutive design of Sokol-Hessner and Schmitz (2002). We use a factorial design that adjusts the densities of individual predators that have different per capita predation rates so as to maintain a constant expected predation rate in all treatments under the null hypothesis of linear intraspecific and additive interspecific predator effects. This general design avoids confounding relative predator frequency with variation in total predation rate and also offers higher statistical power to detect synergistic predation effects under some conditions. In the experiment reported here, we employ a factorial design that exploits prior knowledge of individual species' predation rates and matches the statistical null hypothesis of the design (no treatment effects) with the ecological null hypothesis of interest (no non-additive effects).

Materials and methods

A modified substitutive design

The general substitutive design for studying whether predators combine to create synergistic effects (e.g., Sokol-Hessner and Schmitz 2002) maintains constant total individual density but varies the relative frequency of predators. For example, a design with two species might have one treatment level with 12 individuals of species A, another with 12 individuals of species B, and a third treatment level that combines 6 individuals of each species. This design is akin to substitutive designs for competition experiments that ask if an individual of species B is equivalent to an individual of species A (Warner et al. 1993; Inouye 2001). The test for a synergistic effect of the predator combination — whether predators exhibit mutual interference or facilitation — is to compare the mortality rate of prey exposed to a combination of both predators with the rates imposed by each predator when alone. This is accomplished via an omnibus analysis of variance and an a priori contrast that com-

pares the average mortality rate in the combination against the averages of the rates seen in the two isolated predator treatment levels.

This design works best when each predator has the same expected effect on the prey when alone. In this case, the statistical null hypothesis, that there is no difference among treatments, is a perfect match to the biological hypothesis, that the predator combination imposes a mortality rate which is no different than that imposed by each individual predator treatment. That is, individuals of the two predator species are perfectly equivalent and combinations of all relative frequencies of the two predators impose the same expected mortality rate on the prey.

If the predator species do not have the same predation rate, then the mortality rate expected in the predator combination, under the null hypothesis of no predator interference or mutual facilitation, is a function of the relative frequencies of each predator species. To see this, suppose that under the experimental conditions and experimental period employed, each individual of species A consumes two prey when only species A is present and each individual of species B consumes four prey when only species B is present. Using our earlier example, a design that used 12 individuals of each species when alone and 100 prey, would create a mortality rate of 0.24 in the "A only" treatment and 0.48 in the "B only" treatment. In a combination of A and B, a 50:50 ratio of the two predators would create an expected mortality rate of 0.36 under the null hypothesis, whereas a 25:75 ratio of A to B individuals would create an expected mortality rate of 0.42. In either case, the statistical null hypothesis being addressed by the omnibus test within the analysis of variance does not match the biological null hypothesis of no synergism. A significant difference among treatments is expected because of the variation in predation rates.

There is a straightforward method around this dilemma, which is to use an a priori contrast within the analysis of variance. The null hypothesis for this contrast is that the mortality rate in the predator combination is a weighted average of the rates exhibited by the individual predator treatments. When the predator combination has a 50:50 ratio of two predators, this is equivalent to contrasting the average mortality rate in the combination against the midpoint between the rates in each isolated predator treatment. This approach, which solves the confounding of relative frequency with different total predation rates, will falter with three or more predators. This is because the contrasts for each pairwise effect are not independent of one another and there is no unambiguous contrast for testing three-way synergism. It can also lack power to detect some pairwise effects; if predator interference or facilitation is mild, the mortality rate in the combination will be within the range bracketed by the isolated predator treatments and the a priori contrast will be weak (Scheffe 1959).

We suggest an alternative design that generalizes to three or more predators and avoids the potential power problem. In this design, we use previous knowledge of each predator's capabilities to adjust the densities of individual predator species and predator combinations to maintain as constant a total predation rate as possible. To continue our example, we would use 12 individuals of predator A when alone and 6 of predator B when alone so that each single-predator treatment

has an expected mortality rate of 0.24. We could use many combinations of species A and B to obtain an expected mortality rate of 0.24; we can either divide the total predation rate equally between predator species or, for ecological realism, the relative numbers of each predator species should be as close to natural relative abundances as possible. The ability to capture realistic relative frequencies of predators without complicating the statistical testing will aid interpretation of the results and is another virtue of this approach. This alternative design may confer more statistical power for detecting subtle effects of predator combinations than a design that is substitutive with density and not predation rate.

Experimental design and execution

We performed a factorial experiment evaluating the effects of three predator species: each species alone, in all pairwise combinations, a three-way combination, and a control treatment with no predators. The prey were *H. cinerea* tadpoles and the predators were the centrarchid fish *Micropterus salmoides* (Lacepède, 1802) (hereinafter bass, mean standard length of 39.2 mm), aeshnid odonate naiads *Anax junius* (Drury, 1773) (hereinafter aeshnid, mean total body length of 32.1 mm), and libellulid odonate naiads *Erythemis simplicicollis* (Say, 1839) (hereinafter libellulid, mean total body length of 12.9 mm). These predators have different foraging patterns: bass are fast moving, open water hunters; aeshnids are sit-and-wait predators that may also actively stalk prey in vegetation, while libellulids perch in vegetation or on the substrate and rarely move.

We manipulated the number of each predator species in each treatment so that the expected predation rate would be approximately the same in all treatments at the start of the experiment. First, we determined the average number of tadpoles consumed by an individual predator in 1 m diameter wading pools in 19-h trials. Each pool contained plastic mesh refuges and was stocked with 1 predator and 20 tadpoles of comparable sizes to those used in the experiment in this study (for complete methods see Gunzburger and Travis 2004). We performed four replicates per predator for these estimates. The estimated predation rates (*H. cinerea* tadpoles consumed/h) were 0.64 ± 0.33 (mean ± SD) for bass, 0.53 ± 0.22 for aeshnid, and 0.12 ± 0.04 for libellulid. Using these predation rates, we chose combinations of predators that would maintain as constant a predation rate as possible across predator treatments of approximately 1.5 tadpoles consumed/h (Table 1). We first set the lowest possible densities of predators in the three-predator treatment by using one individual of the predator with the highest predation rate (bass), and then set all the remaining treatments to be as close to this expected predation rate as possible without having a higher predation rate (Table 1). If the combined effect of predator individuals was linear within species and additive across species, the total expected predation rate for the treatments would range only between 1.3 and 1.8 prey/h (Table 1). However, this predicted predation rate does not take into account changes in predation rate owing to prey depletion over time and the presence of alternate prey. In treatments with multiple predators the contribution of each predator species to the total predation rate was approximately equal. This design maximized the uniformity of expected predation rates across all treatments while using whole predator individuals

Table 1. Mesocosm experimental design evaluating the effects of three predator species on *Hyla cinerea* tadpoles.

Treatment	Predators	Expected total predation rate*
Control	0	0
Aeshnid (A)	3	1.6
Libellulid (L)	13	1.6
Bass (B)	2	1.3
Aeshnid + libellulid	2 A, 5 L	1.7
Aeshnid + bass	2 A, 1 B	1.7
Bass + libellulid	1 B, 9 L	1.7
Aeshnid + bass + libellulid	1 A, 1 B, 5 L	1.8

*The expected total predation rate (number of tadpoles consumed/h) for each treatment assumes that all predator effects are additive.

and maintaining equality among the expected contributions of each predator in the combinations. We performed three replicates of each of the eight treatments.

Predation experiments were performed using 24 cattle trough mesocosms located at the Florida State University greenhouse facility in Tallahassee, Leon County, Florida. These mesocosms were oblong troughs (183 cm long × 61 cm wide × 61 cm deep) with sand added to one half of the trough to provide a gradual depth gradient from 50 to 5 cm deep. Mesocosms were filled with well water to a total volume of 568 L, 1 L of filtered pond water, and an inoculum of zooplankton and refuges were added (14 refuges constructed of sliced plastic garbage bags to resemble aquatic vegetation and 7 short PVC tubes), and mesocosms were covered with tightly fitting windowscreen covers. The mesocosms used for this experiment were larger and differed in depth and gradient from the wading pools used for the preliminary trials establishing predation rates described above. However, if these differences in mesocosm design have an effect on the predation rates of these predators, it is likely that all predators were affected similarly. A previous study evaluating the influence of experimental scale on the results of a tadpole predation experiment showed that variation in mesocosm depth (water volume) did not significantly affect mortality rate of tadpoles in response to predation (Gascon and Travis 1992).

Hyla cinerea egg clutches were collected on 9 July 2002 from five amplexed pairs from Harriman Pond (30.476°N, 84.252°W) in Leon County, Florida. Eggs from all clutches were mixed and tadpoles were raised in aquaria in the greenhouse. On 16 July 2002, 130 tadpoles were selected haphazardly from the aquaria and added to each mesocosm. Total length of tadpoles was 7.0 ± 0.9 mm at the beginning of the experiment. To serve as alternate prey for the predators, 10 adult female *Heterandria formosa* Girard, 1859, a small fish found in most permanent water habitats in northern Florida (Leips and Travis 1999), were also added to each mesocosm. Survival of alternate prey was not monitored.

Predators were collected using seines and dipnets from various aquatic habitats and maintained in the greenhouse in plastic tubs (aeshnids and libellulids) and aquaria (bass) and fed *H. cinerea* tadpoles and *H. formosa* fish ad libitum before the experiment. Observations of feeding behavior of the three predator species indicated that the active foraging of aeshnids and bass may result in a pulse of high tadpole mor-

Fig. 1. Cumulative percent mortality of *Hyla cinerea* tadpoles in each treatment at (*a*) the first census, day 3 and (*b*) the second census, day 6. Values are means ± SE and are shown for three replicates of each treatment, except for libellulid which consisted of two replicates. L, libellulid; B, bass; A, aeshnid. Horizontal lines join treatments with mean values that were not significantly different (ANOVA, $p > 0.05$).
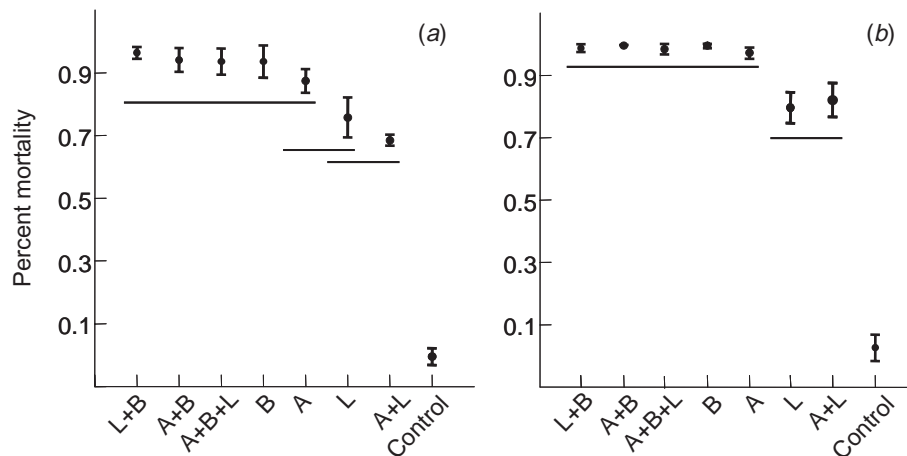
tality at the beginning of an experiment. To reduce potential bias from an initially high foraging rate, we added chemical cues (0.5 L of water from the bass holding tanks and the tubs in which aeshnids were housed) to the troughs in which these predators were to be added on 17 July 2002 (Werner and McPeek 1994; Werner and Anholt 1996). On 18 July 2002, after tadpoles had acclimated in the troughs for 2 d, predators were added to each trough.

We censused troughs every few days throughout the experiment to monitor mortality and habitat use of *H. cinerea* tadpoles and predators. We suspected that tadpoles might use the shallow portion of the mesocosm as a refuge from predation. Although we observed tadpoles using the artificial plant refuges, we did not quantify use of these refuges because algal growth in many tanks prevented accurate quantification of the number of tadpoles visible outside of these refuges. In addition, during censuses some tadpoles would move short distances into the refuges in response to our approach to the mesocosm. During censusing, a Plexiglas® divider was placed across the middle of the trough to separate the deep and shallow ends of the mesocosms. All tadpoles and predators were removed from each section of the trough using a small aquarium net. Tadpoles in the shallow and deep section of each trough were counted and returned to the appropriate section of the trough. Predators were counted, we replaced any missing or dead predators, and released back into troughs at least 20 min after tadpoles had been returned to the mesocosms. Some natural mortality of predators may have occurred, but for purposes of our analysis we assume all predator deaths were due to intraguild predation.

Statistical analyses

We used tadpole mortality values at the first two censuses, 3 and 6 days after the addition of predators, to assess the effects of the experiment. These data offer unbiased estimates of comparative survival rates; at the third and subsequent census, predators eliminated tadpoles from some replicates, creating truncated distributions of survival that no longer reflect relative rates. Because predation is highest on *H. cinerea* tadpoles at small sizes, this statistical issue is unlikely to cre-

ate a misleading ecological result (Gunzburger and Travis 2004). It is unlikely that a longer experiment would indicate different results. One replicate in the libellulid treatment was a consistent statistical outlier and it was removed from the analysis. Mortality data were normally distributed and did not require transformation.

If all predator effects were linear within predator species and additive across predator species, then the mortality of tadpoles should not differ across predator treatments. We examined this hypothesis for each census by performing an analysis of variance (ANOVA) on the percent mortality of tadpoles across the eight treatments (including controls); we made post hoc mean comparisons with the Tukey method, making Bonferroni corrections for multiple comparisons. Next, to determine if *H. cinerea* tadpoles alter their use of water depth in response to predator treatments at the first census, we compared the proportion of surviving tadpoles in shallow water to the proportion of all predators in shallow water across all predator treatments using linear regression analysis. If tadpoles avoid predators spatially, then we expected the proportion of tadpoles in shallow water and the proportion of predators in shallow water to be negatively correlated. To determine if tadpoles consistently avoid one predator species across all treatments in which it occurred, regardless of the other predators present, we also compared the proportion of tadpoles in shallow water with the proportion of individuals of each predator species in shallow water across all treatments in which each predator occurred.

Results

Hyla cinerea tadpole mortality varied across predator treatments in both the first and the second censuses (Fig. 1). In the first census, mortality was significantly lower in control treatments than all other treatments, and aeshnid + libellulid and libellulid treatments generally had lower mortality than the remaining five treatments, which had similar, high mortality rates (ANOVA, $F_{[7,15]} = 150$, $p < 0.001$; Fig. 1*a*). This pattern held through the second census, which presented three levels of tadpole mortality: low in control

Table 2. Summary of intraguild predation in the multiple predator mesocosm experiment showing the number of dead or missing predator individuals for each of three replicates for each census.

Treatment	First census, replicate			Second census, replicate		
	1	2	3	1	2	3
Aeshnid (A)	2	—	—	—	—	—
Bass (B)	—	1	—	—	—	—
Libellulid (L)*	1	1		1	—	
Aeshnid + bass	—	1 A	—	1 A	—	—
Aeshnid + libellulid	1 A, 3 L	1 L	5 L	2 L	3 L	5 L
Libellulid + bass	2 L	3 L	4 L	3 L	2 L	2 L
Aeshnid + bass + libellulid	1 L	3 L	1 L	3 L	2 L	1 L

Note: A dash indicates that no predators were missing or dead in the given replicate.

*For this treatment, a replicate was removed from the analysis.

Fig. 2. Proportion of surviving *H. cinerea* tadpoles in each treatment (A, aeshnid; L, libellulid; B, bass) in the shallow portion of the mesocosm at the first census versus proportion of total predator individuals in the shallow portion.
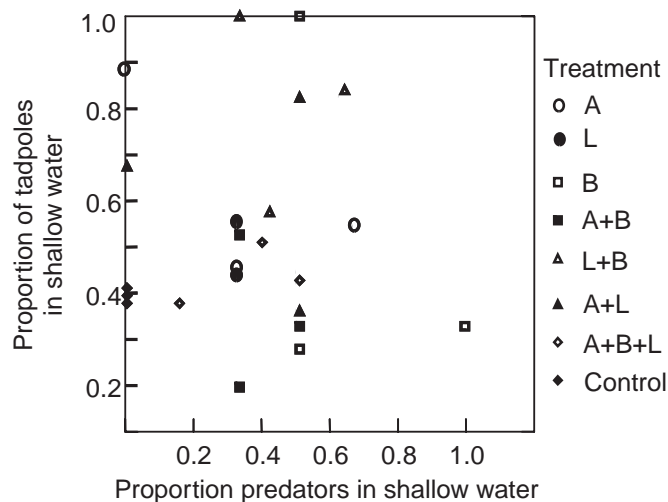
treatments, intermediate in libellulid and aeshnid + libellulid treatments, and high in all five remaining treatments (ANOVA, $F_{[7,15]} = 240$, $p < 0.001$; Fig. 1*b*). It is possible that there were additional differences among treatments in tadpole mortality during the first 2 days of the experiment that were not detected at the first census.

Hyla cinerea tadpoles did not demonstrate any tendency to avoid predators by using different areas of the trough mesocosms. There was no relationship between the proportion of tadpoles in shallow water and the proportion of predators in shallow water across predator treatments (Fig. 2). In addition, with the exception of the control treatments, there was no tendency for replicates of the same treatment to have similar tadpole and predator distributions throughout the trough mesocosm (Fig. 2). Tadpole distribution was not correlated with either bass or libellulid distribution; however, there was a marginally significant negative relationship between the proportion of aeshnids in shallow water and the proportion of tadpoles in shallow water ($r^2 = 0.26$, $p = 0.09$).

Intraguild predation was observed in all predator treatments (Table 2). Libellulids suffered the highest rate through predation from bass, aeshnids, and other libellulids. Aeshnids seemed to cause the highest mortality rate of libellulids,

in aeshnid + libellulid treatments an average of three out of five libellulids were replaced during each census. In one replicate of the aeshnid + libellulid treatment in both censuses, all five libellulids were killed (Table 2). Aeshnids consumed other aeshnids and, in a separate experiment using the same mesocosm setup, aeshnids also ate bass (M.S. Gunzburger, unpublished data).

Discussion

Hyla cinerea tadpole mortality rate in most predator treatments was similar to the overall expected tadpole mortality rate (1.6 tadpoles/h, 88% mortality by the first census; Table 1, Fig. 1). Thus, for many of the predator species combinations, there is no evidence of non-additive effects. Tadpole mortality was significantly lower in aeshnid + libellulid and libellulid treatments. Combinations of multiple predator species, which varied considerably in predation rate and foraging mode, might be expected to generate predation rates on *H. cinerea* tadpoles that exceeded the rates produced by single predator species (Martin et al. 1989). In fact, treatments with combined predator species did not tend to show higher mortality rates overall than the single predator species treatments (Fig. 1). Other experiments evaluating the interactions of multiple predators with different foraging modes have shown that such predator combinations do not always elicit greater than additive effects on prey (Krupa and Sih 1998; Sokol-Hessner and Schmitz 2002).

Interactions among predators may influence the effects of multiple predators on prey (Spiller and Schoener 1990). Direct effects of predators on each other were prevalent in this experiment, intraguild predation was observed in all predator combinations. Although it is possible that intraspecific predator interactions resulted in the lower *H. cinerea* tadpole mortality rate in the aeshnid + libellulid treatment, intraguild predation is a more likely explanation. Overall mortality of libellulids at the first census in the three libellulid + aeshnid treatments was 60%, nearly twice the mortality rate in any other treatment in which libellulids were present, and much higher than libellulid mortality in the libellulid only treatment (8%) (Table 2). Aeshnids apparently reduced their predation rate on tadpoles by consuming libellulids, thus further reducing the total predation rate on tadpoles in this treatment (Table 2). Any surviving libellulids may have significantly reduced their foraging behavior to avoid detection and predation by aeshnids. Libellulids also suffered high mortality

in the libellulid + bass treatment (33% mortality at the first census; Table 2), yet tadpole mortality was not significantly lower in this treatment as it was in the aeshnid + libellulid treatment. The initial number of libellulids in the bass + libellulid treatment was higher than in the aeshnid + libellulid treatment (Table 1), and at the first census there were, on average, still three times as many libellulids in the bass + libellulid treatment (6) as in the aeshnid + libellulid treatment (2). Thus the predation rate on libellulids with bass may have been insufficient to result in reduced predation rate on tadpoles. Predator interactions may also explain the lower mortality of tadpoles in the libellulid treatment. Although intraspecific predation of libellulids was relatively low (Table 2), the high density of libellulids in each mesocosm may have resulted in a high encounter rate that may have decreased their foraging rate on tadpoles.

Prey may adopt compensatory defenses, such as antipredator behavior, that reduce predation by more than one predator type (Krupa and Sih 1998). In this experiment, we evaluated the use of habitat as a potential spatial antipredator avoidance behavior. *Hyla cinerea* tadpoles' use of shallow habitat varied considerably across treatments (Fig. 2). However, there was no significant evidence that tadpoles were avoiding predators by moving to another section of the trough, a result that might be expected when both predators and prey are mobile (Formanowicz and Bobka 1989). Other tadpole behaviors including reducing activity level and increasing use of refuges may increase tadpoles survival from predation (Werner 1991), but these behaviors could not be accurately quantified for this experiment.

It is possible that the similar effects on tadpole mortality of all of the treatments that contain bass actually reflect an overwhelming effect of bass predation. One might argue that bass are such effective predators that the addition of any other predators will have too small an effect to be detected by an experiment of this size. If this were the case, the equivalent survival values found when only bass were present and when bass were present with other predators actually reflects a non-additive, "risk reducing" effect (sensu Sih et al. 1998). However, *H. cinerea* tadpole mortality with bass alone was not significantly different than that with aeshnids alone and the average values were not very different, regardless of one's reliance on the level of statistical significance. That aeshnids alone can eliminate almost as many tadpoles as bass alone and that the combination does not produce an obvious increase in tadpole mortality argue against our results reflecting merely an overwhelming effect of a single bass in the combined treatments.

Our design, which attempts to maintain a constant attack rate instead of a constant density, has an additional fortuitous feature. The relative predator densities that we used in our mesocosms are similar to their relative densities in nature (Gunzburger and Travis 2004). In nature, the predators with the highest per capita effect on *H. cinerea* tadpoles, the centrarchid fish, are much less abundant than predators such as odonates that have lower per capita effects. In this light, the effects of predator combinations that we detected here seem likely to be manifested in natural systems. Experiments on predator combinations that use an additive density series must be interpreted carefully; synergistic effects in predator combinations could be due to having combined total densi-

ties that exceed those ever manifested in nature, which might compromise the inference that such effects play important ecological roles. There is a parallel danger with substitutive designs when predator species differ widely in attack rates.

Our results offer insight into assessing predation risk for these *H. cinerea* tadpoles across habitats that vary markedly in total predator density and composition. In our previous work (Gunzburger and Travis 2004), we assessed the importance of predator species based on their abundance and relative per capita predation rates. That assessment led us to conclude that, for *H. cinerea*, temporary ponds represented a riskier habitat than permanent ones, a conclusion at odds with the prevailing view that permanent ponds exhibit greater levels of predation risk for tadpoles. Here we found little evidence for synergistic effects of predator combinations at realistic relative densities, thus for *H. cinerea*, tadpole mortality rates tend to increase linearly with predator density.

Acknowledgements

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